Cell Host & Microbe
Previews

Transgenic Parasites: Improving Our Understanding of Innate Immunity to Malaria

Rick M. Fairhurst[1,*]
[1]Laboratory of Malaria and Vector Research, NIAID/NIH, Bethesda, MD 20892, USA
*Correspondence: rfairhurst@niaid.nih.gov
DOI 10.1016/j.chom.2007.07.007

Clinical immunity to *Plasmodium falciparum* malaria takes years to develop and is never complete. One explanation for these observations is that antigenic variation enables malaria parasites to evade humoral immunity; another is that *P. falciparum* induces immune dysregulation, which inhibits the development of protective cellular immunity. Research described by D'Ombrain et al. in this *Cell Host & Microbe* issue probes how the parasite's main virulence factor *Pf*EMP-1 might significantly alter human innate immune responses.

Plasmodium falciparum erythrocyte membrane protein-1 (*Pf*EMP-1) is a clonally variant, multidomain ligand expressed on the surface of parasite-infected red blood cells (iRBC). Most *Pf*EMP-1 variants mediate the adherence of iRBC to CD36 receptors expressed on microvascular endothelial cells. By this process, iRBC avoid clearance from the blood stream by the spleen and multiply to sufficiently high numbers to cause symptomatic disease. While iRBC adherence to microvessels is pathogenic, the expression of CD36 on macrophages and dendritic cells (DC) suggests that *Pf*EMP-1 might also exert immunoregulatory and/or immunopathogenic effects. Recent in vitro studies have shown that macrophages can ingest nonopsonized iRBC in a *Pf*EMP-1- and CD36-dependent manner, which may be important in the phagocytic clearance of iRBC from nonimmune hosts (McGilvray et al., 2000). Another study suggested roles for *Pf*EMP-1 and CD36 in the parasite's inhibition of DC function (Urban et al., 1999). However, results obtained recently from transgenic parasites that lack *Pf*EMP-1 and parasites that express a non-CD36-binding *Pf*EMP-1 variant indicate that neither *Pf*EMP-1 nor CD36 are involved in iRBC-DC interactions (Elliott et al., 2007).

D'Ombrain et al. have now used transgenic parasites to explore another potential immunoregulatory function of *Pf*EMP-1, as described in this issue of *Cell Host & Microbe* (D'Ombrain et al., 2007b). These and other investigators previously reported that iRBC activate malaria-naive peripheral blood mononuclear cells (PBMC), as indicated by the rapid production (within 24 hr) of IFN-γ from γδ-T cells, NK cells, and naive αβ-T cells (D'Ombrain et al., 2007a). Conceivably, the innate production of IFN-γ could help to reduce parasite replication by activating macrophages to ingest parasites in the spleen. To determine whether *Pf*EMP-1 might specifically modulate this IFN-γ response, these investigators used a pair of isogenic *P. falciparum* lines that differ only in the presence or absence of *Pf*EMP-1. They found that iRBC lacking *Pf*EMP-1 induce much higher levels of IFN-γ than iRBC expressing *Pf*EMP-1, suggesting that *Pf*EMP-1 suppresses the innate IFN-γ response to iRBC. Most of the IFN-γ was produced by γδ-T cells, with NK cells and naive αβ-T cells producing small amounts of this cytokine. Similar results were obtained with a parasite line that expresses a *Pf*EMP-1 variant which does not bind CD36. Thus, it seems that *Pf*EMP-1 suppresses IFN-γ production from malaria-naive PBMC by a mechanism that does not involve CD36-expressing macrophages or DC.

Based on these and other data, D'Ombrain et al. are now able to propose a more detailed model of innate immunity to malaria (Figure 1). When iRBC rupture as part of their life cycle, parasite products (e.g., GPI and hemozoin) bind to Toll-like receptors and other pattern recognition receptors on macrophages and DC. These antigen-presenting cells display additional parasite antigens to various immune effector cells. One example is the presentation of phosphoantigens by plasmacytoid DC to γδ-T cells. The rapid IFN-γ responses by γδ-T cells, NK cells, and naive αβ-T cells are each suppressed by *Pf*EMP-1. Since CD36-expressing macrophages and DC are not involved in this suppression, the authors propose that *Pf*EMP-1 might exert its suppressive effects through a common receptor on NK and T cells, such as an inhibitory NK receptor or another constitutive negative regulator of leukocyte activation (Figure 1, bottom panel).

If this model operates in vivo, two interpretations can be made. If the early IFN-γ response activates macrophages to clear parasites by phagocytosis, then the suppression of this cytokine surge by *Pf*EMP-1 might improve parasite survival—an outcome presumably detrimental to the host. On the other hand, reduced levels of IFN-γ might ameliorate disease severity, as high levels of this cytokine have been associated with disease severity in animal models and human malaria studies (Hunt and Grau, 2003). One might then hypothesize that *Pf*EMP-1 reduces IFN-γ levels just enough to allow parasites to survive in reasonable numbers and also reduce the chance of causing severe, life-threatening disease in the host. Like IFN-γ, TNF and other cytokines have been plausibly implicated as having both antiparasitic and immunopathogenic effects during

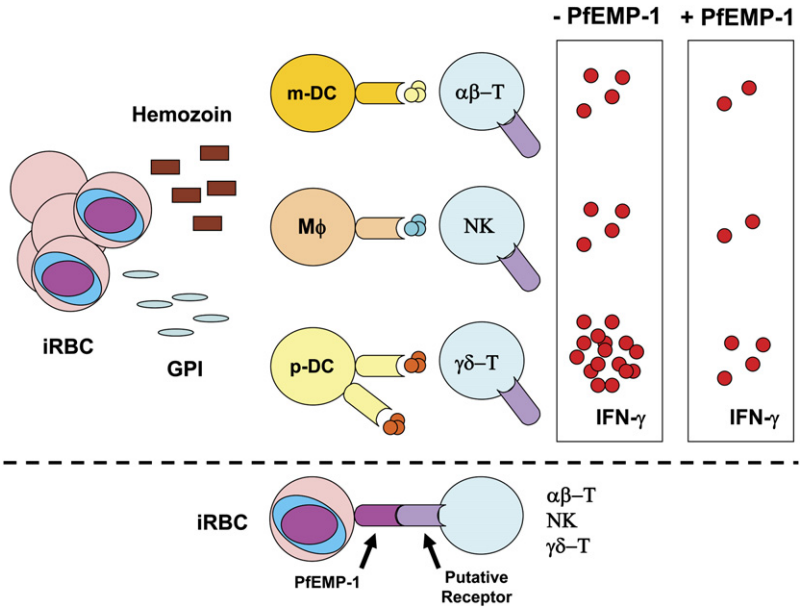

Figure 1. A Model of Innate Immunity to *P. falciparum* Malaria

Top panel: Hemozoin and GPI from iRBC activate macrophages and both myeloid and plasmacytoid DC. These antigen-presenting cells display additional parasite antigens to naive-T and NK cells. By this process, transgenic iRBC that lack *Pf*EMP-1 stimulate the production of large amounts of IFN-γ mostly from γδ-T cells. iRBC that express *Pf*EMP-1 induce lower levels of IFN-γ from naive-T and NK cells in a CD36-independent manner, indicating that macrophages and DC are not involved in IFN-γ suppression. Instead, the authors propose that *Pf*EMP-1 suppresses IFN-γ production directly by binding to a putative receptor that is common to naive αβ-T, NK, and γδ-T cells (bottom panel).

episodes of disease (Clark and Cowden, 2003). Some innate immune responses thus seem to work as a two-edged sword in producing cytokine levels that are pathogenic and protective in vivo.

Further work to clarify *Pf*EMP-1-mediated effects on immune cells and their relevance to antimalarial immunity in vivo are clearly needed. In some of their experiments, D'Ombrain et al. used a parasite line containing populations of parasites expressing multiple CD36-binding variants of *Pf*EMP-1 (D'Ombrain et al., 2007b). Whether clonal variants of *Pf*EMP-1 expressed by naturally circulating parasites vary in their ability to suppress IFN-γ is not yet determined. Since *Pf*EMP-1 variants differ in their affinity for CD36, it is conceivable that *Pf*EMP-1 variants might also differ in their affinity for putative receptors on T and NK cells. Also, *Pf*EMP-1 variants that bind ICAM-1 (the receptor for iRBC in cerebral microvessels) could perform very differently in PBMC stimulation assays, as most leukocytes express ICAM-1. The possibility that host genetic polymorphisms might influence the development of innate immunity can now be considered a promising area of research. Recent data have shown that hemoglobin C, a hemoglobin variant associated with malaria protection in West Africa, markedly reduces the amount of *Pf*EMP-1 on the iRBC surface (Fairhurst et al., 2005). It is possible that some naturally-selected variants of hemoglobin and erythrocytes lessen *Pf*EMP-1-mediated suppression of IFN-γ, thereby interfering with the ability of the parasite to control host defense mechanisms against infection.

Determining whether *Pf*EMP-1-mediated suppression of IFN-γ might influence parasite survival and disease-controlling immunity in malaria-experienced individuals will not be easy. In Papua New Guinea, where Schofield and colleagues are conducting a longitudinal cohort study, it is likely that naturally-circulating parasites express myriad *Pf*EMP-1 variants and that study participants will have varying titers and repertoires of anti-*Pf*EMP-1 antibodies. These antibodies could interfere with the iRBC-leukocyte interactions that promote IFN-γ suppression. Human populations of Papua New Guinea also carry extremely high frequencies of RBC-related polymorphisms known to influence disease severity, such as those associated with ovalocytosis and alpha-thalassemia (Allen et al., 1997; Genton et al., 1995). Accounting for various confounders of *Pf*EMP-1-mediated events while maintaining a focus on activated γδ-T cells and IFN-γ will be an immense challenge, but one well worth the effort.

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